Survival of Shiga toxin-producing and generic *Escherichia coli* during ripening of semi-hard raw milk cheese

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STEC in raw milk cheese

• From cattle to milk to cheese

• Raw milk cheese associated STEC outbreaks were reported\(^1\)

• STEC prevalence in raw milk cheese in Switzerland
  • detection rate (PCR): 5.7 % of 1,502 raw milk cheese samples\(^2\)

• Higher risk potential recognized for\(^3\)
  • soft and semi-soft cheeses
  • uncooked cheeses

Spiking of semi-hard raw milk cheese

- Three STEC and two generic *E. coli* strains
  - strains isolated from raw milk cheese
  - each strain is quantified separately*
  - two spiking levels (10\(^1\) and 10\(^3\) CFU/ml)
- Semi-hard raw milk cheese similar to Swiss type
  - two cheese types (cooking temperatures: 40°C and 46°C)
  - two cheeses are produced from one batch of 50 l spiked raw milk
  - two replications of each combination
- Sampling during production and 16 week ripening period

* selectiv media based on inherit properties (e.g. sugar fermentation and antibiotic resistances)
Cheese production

- Raw milk used tested negative after enrichment for presence of
  - target strains
  - $stx$ genes
- Physicochemical parameters
  - in accordance with cheese recipe
  - similar in spiked and unspiked cheeses
  - acidification stronger in 40°C cooked cheeses
    - (pH minima at 5.0 and 5.2)
- Additional flora incl. starter cultures similar in all cheeses
### Strain characteristics

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Virulence factors</th>
<th>RpoS-phenotype</th>
<th>Thermal inactivation</th>
<th>Oxidative AR&lt;sup&gt;a&lt;/sup&gt; system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>stx&lt;sub&gt;1&lt;/sub&gt; stx&lt;sub&gt;2&lt;/sub&gt; eae&lt;sup&gt;b&lt;/sup&gt; hlyA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(catalase test)</td>
<td>(55 °C, 15 min)</td>
<td>(Survival, 2 h, pH 2.5)</td>
</tr>
<tr>
<td>K356</td>
<td>O2:H27</td>
<td>- + - +</td>
<td>+</td>
<td>- 1.52 log&lt;sub&gt;10&lt;/sub&gt;</td>
<td>5.2 %</td>
</tr>
<tr>
<td>K303</td>
<td>O9:H21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>- - - -</td>
<td>-</td>
<td>- 1.89 log&lt;sub&gt;10&lt;/sub&gt;</td>
<td>0.6 %</td>
</tr>
<tr>
<td>N09-1208</td>
<td>O26:H11</td>
<td>+ - + +</td>
<td>+</td>
<td>- 1.90 log&lt;sub&gt;10&lt;/sub&gt;</td>
<td>7.9 %</td>
</tr>
<tr>
<td>K331/4</td>
<td>O91:H21</td>
<td>+ + - +</td>
<td>+</td>
<td>- 1.78 log&lt;sub&gt;10&lt;/sub&gt;</td>
<td>12.6 %</td>
</tr>
<tr>
<td>FAM21843</td>
<td>O178:H12</td>
<td>- - - -</td>
<td>+</td>
<td>- 0.04 log&lt;sub&gt;10&lt;/sub&gt;</td>
<td>27.6 %</td>
</tr>
</tbody>
</table>

<sup>a</sup>Acid resistance, <sup>b</sup>intimin, <sup>c</sup>hemolysin A, <sup>d</sup>phenotypically non-motile.

K356: serotype that showed high prevalence during monitoring program<sup>1</sup>

K303: potential defect in stress response

N09-1208: serotype belonging to the „gang of five“

K331 / 4: serotype associated with severe disease

FAM21843: high stress resistance to acidic and heat stress

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1) Zweifel <em>et al.</em> 2010
Increase of \textit{E. coli} during production

- Increase of app. $3.5 \log_{10}$ CFU/ml observed at both spiking levels
- „Entrapment“ of the bacteria in the curd (app. factor 10)
- Growth of the \textit{E. coli} strains
Survival of *E. coli* (low spiking level)

- Significant differences between the strains in both cheese types
- In six of the 16 cheeses STEC present at ≥ 10 CFU/g at the end of ripening
- Detection of *stx* after enrichment in all but one sample after 16 weeks

Average counts of the *E. coli* strains in 40°C cooked cheeses

(no significant differences were observed between the two cheese types)
Survival of *E. coli* (high spiking level)

- Significant differences between the strains in both cheese types
- Detection of *stx* after enrichment in all but one sample after 16 weeks

Average counts of the *E. coli* strains in 40°C cooked cheeses

(no significant differences were observed between the two cheese types)
**Summary**

- Significant differences in survival of the *E. coli* strains in raw milk cheese
- Generic *E. coli* survive in higher counts than the STEC strains
- In six of 16 cheeses made at low spiking level STEC were present at ≥ 10 CFU/g at the end of ripening

Even low STEC counts in raw milk cheese are a matter of concern due to the low infectious dose of highly pathogenic STEC